suitability for use as a biomarker in DMOAD development. If successful, these methods could both accelerate the early evaluation of new molecules and demonstrate preservation on cartilage composition to support regulatory submission for licensure.

6

SERUM AND URINE BIOCHEMICAL MARKERS FOR USE IN OSTEOARTHRITIS CLINICAL TRIALS

Joanne M Jordan

Using radiographic outcomes, clinical trials of potential disease modifying interventions for development and progression of osteoarthritis, typically last 2 to 3 years. Such trials are expensive, difficult to perform, and subject to biases from attrition and other methodologic problems. Inability to accurately identify individuals likely to progress, for selection into a disease-modification trial, can limit statistical power if insufficient numbers of individuals develop the outcome under study. More sensitive imaging measures of OA outcome, such as magnetic resonance imaging, have been discussed. Biochemical markers, measured in serum or urine, are also under investigation to substitute for radiographic or other OA outcomes that might require a longer trial duration, and to help identify individuals whose OA is likely to develop or progress quickly. If a biomarker or panel of biomarkers, in conjunction with clinical or other easily obtainable measures, were able to identify individuals at high risk of rapid OA progression or were to predict response to intervention early, then the duration, number of study participants, and expense of clinical trials could be decreased and efficiency enhanced.
In 2003, the NIH/NIAMS established The Osteoarthritis Biomarkers Network as an international consortium of five sites engaged in innovative approaches to biomarker development and testing in OA. This consortium is working to develop a classification scheme for OA biomarkers that could provide a consistent terminology and framework to facilitate research, development, and validation of biomarkers. This classification scheme, still under development, proposes five categories of markers: 1) Investigative; 2) Diagnostic; 3) Burden of disease, encompassing measures of disease activity and damage; 4) Prognostic, and 5) Efficacy (and toxicity) of intervention. These classification categories are not mutually exclusive, nor do they necessarily imply that a marker must move serially through each of the categories in its development. Markers can fulfill criteria in more than one category. This proposed classification scheme, criteria for the categories, and examples of current serum and urine biomarkers in each category will be discussed.

7

GRADING SYSTEM FOR MOUSE OSTEOARTHRITIS

Qian Chen, Sonya S Glasson

In recent years, knockout and transgenic mouse has become a widely used model to determine the contribution of a specific gene to the development of a disease *in vivo*. Many genes have been implicated in the pathogenesis of osteoarthritis (OA). However, unlike the OA occurring in human joint cartilage, there is no standardized grading system to evaluate the progress of OA in mouse joint cartilage. The lack of a uniform mouse OA grading system has made comparison of different mouse OA models difficult. The goal of this session is to gather the researchers who are working in the mouse OA field, and develop a mouse OA grading standard that is accepted by the research community. Such standardized mouse OA grading system will become a guideline to assess the severity of OA in mouse joint cartilage. This session will consist of three parts.

8

IDENTIFICATION OF TARGETS THROUGH HISTOLOGIC EVALUATION OF OSTEOARTHRITIS IN KNOCK OUT MICE

Sonya Glasson

Introduction: There are no disease-modifying osteoarthritis drugs (DMOADs) currently available for the treatment of OA. In order to identify which enzymes or cytokines are important *in vivo*, studies were performed in knock out (KO) mice that were challenged in a surgical instability model. Those KOs showing a decrease in OA severity, following histologic evaluation would be targets for the development of selective inhibitors.
Methods: In an IACUC-approved protocol, 12 different 10-week old knock-out (KO) and strain-specific wild-type (WT) mice had surgical instability induced in their right knees via destabilization of the medial meniscus (DMM). IL-1β (B10.RIII WT), MMP-3 (B10.RIII WT), COX-1, COX-2 (B6;129) KOs were from Taconic. Jak-3 (C57BL6) and MMP-9 KO (FVB/N WT) mice were from Jackson Laboratories (Bar Harbor, ME). MMP-12, cPLA$_2\alpha$ (C57BL/6 WT), MK2 (DBA), ADAMTS-4, -5, 4/5 (129SvEv WT) KO mice were from Wyeth. Ten males per experimental group were sacrificed 8 weeks post-operatively. Knees were fixed in 4% paraformaldehyde, decalcified, stained with Safranin-O and scored by 2 blinded observers using a modified semi-quantitative system (Chambers et al. 1997) where a higher score reflects greater OA severity. Histologic scores were obtained from all four quadrants of the joint (MFC, MTP, LFC, LTP) across 12-16 levels spaced at 80 μm intervals. Results were expressed as a sum of all scores and could also be expressed as maximal scores across the entire joint or within a specific location (MTP).
Results: Severity of OA varied greatly depending on the WT strain, with the most severe OA in the 129/SvEv strain, followed by C57B10.RIII, C57BL/6, FVB/N and DBA. There were no differences between MMP-3, COX-1, COX-2, Jak-3, MMP-12, cPLA$_2\alpha$, or ADAMTS-4 KO mice and their respective wild-type controls. The MMP-9 and MK2 KO mice had higher scores, reflecting more severe OA, than their WT controls. The scores in the IL-1β, ADAMTS-5 and ADAMTS-4/5 KOs were significantly decreased compared to controls, using any variation of the scoring scheme, although greater sensitivity was observed using the summed method.
Discussion: Histologic evaluation of mouse knee joints following surgical instability is sensitive to the effects of strain and to disease modification from IL-1β or ADAMTS-5 deletion. More severe OA could be observed in MMP-9 or MK2 deficient mice, suggesting that these enzymes could play a protective role, or that other enzymes are up-regulated to compensate. The deletion of the majority of genes examined showed no impact on the progression of OA, suggesting that these have an insignificant role in OA progression.

9

DESIGN AND CONDUCT OF CLINICAL TRIALS IN OSTEOARTHRITIS

Marc C Hochberg

The Osteoarthritis Research Society International has published recommendations for the conduct of clinical trials in patients with hip or knee osteoarthritis (1) and is developing similar recommendations for patients with hand osteoarthritis. This session will review the design of randomized clinical trials focusing on 1) types of trials (superiority, equivalence, non-inferiority), 2) types of controls (placebo, active treatment), 3) inclusion criteria (including use of American College of Rheumatology [ACR] classification criteria for case definition), 4) exclusion criteria, 5) types of in-

terventions (pharmacologic and non-pharmacologic), 6) types of outcome measures (self-reported, performance-based, examination, imaging), and 7) considerations in the statistical analysis of data. Participants will complete an exercise that designs a clinical trial for symptom and structure modification in patients with knee osteoarthritis.

10

ROLE OF MATRIX METALLOPROTEINASES IN CARTILAGE AND BONE DURING SKELETAL REMODELING

Stephen M Krane

Matrix metalloproteinases (MMPs) are expressed in cartilage during embryonic development and later during remodeling. Mmp9, although not a collagenase is expressed early in embryogenesis during endochondral ossification and *Mmp9* deficiency in mice results in abnormalities that affects growth plates. Mmp13 (collagenase-3), a highly expressed collagenolytic MMP in cartilage and in developing and remodeling bone, also has a role in the joint tissue destruction that is a major feature of various forms of human arthritis. We have targeted a null mutation in mouse *Mmp13* that resulted in a profound embryonic and adult skeletal phenotype characterized by abnormal growth plates and delayed ossification. During embryonic development at the earliest stage examined, *Mmp13*$^{-/-}$ mice had growth plates in long bones almost double in length, accounted for by increases in the zone of hypertrophy. Mmp13, produced by chondrocytes but not by osteoclasts/chondroclasts, is particularly effective in proteolysis of type II compared to type I collagen. Using antibodies that detect epitopes in the specific proteolytic fragments, we obtained evidence for Mmp13 cleavage of type II collagen *in vivo* in wt mice, but not in *Mmp13*$^{-/-}$mice. It is thus unlikely that other Mmps compensate for the loss of Mmp13 function in cartilage. The delay in ossification, so prominent in 15.5 dpc *Mmp13*$^{-/-}$ embryos, is largely transient and older *Mmp13*$^{-/-}$ mice have *increased* bone deposition. *Mmp8* is also expressed in newborn *Mmp13*$^{-/-}$ and wt skeletons but *Mmp*$^{+/-}$ mice have normal skeletons. Type X collagen is also a substrate for MMP1 and Mmp13. The area of type X collagen deposition was significantly increased in growth plates from *Mmp13*$^{-/-}$mice, consistent with decreased proteolysis. Increased synthesis of type X collagen could also contribute. Based on our results, Mmp13 has a critical role in regulating events in the growth plate beginning in embryonic development. Deficiency of Mmp13 with failure to normally resorb collagens in the cartilage ECM profoundly affects cellular activities that underlie differentiation of hypertrophic chondrocytes that persist in newborn and adult mice. The phenotype of adult *Mmp13*$^{-/-}$mice with increased length of growth plates, increased numbers of chondrocytes and distortion of alignment of the rows of chondrocytes thus has features of a chondrodysplasia. Articular cartilage is also affected. It is pertinent that a form of human chondrodysplasia, the Missouri variant of spondyloepimetaphyseal dysplasia is caused by a mutation in *MMP13* that results in misfolding, intracellular degradation and MMP13 deficiency. Human mutations in *MMP2* also result in a skeletal phenotype (nodulosis, osteolysis and arthropathy) distinct from that in *MMP13* mutations. Bone is abnormal in *Mmp2*-null mice but the mice do not show other features of the human mutation.

11

INTEGRIN-SYNDECAN CO-OPERATION GOVERNS THE ASSEMBLY OF SIGNALLING COMPLEXES DURING CELL MIGRATION

Martin J Humphries

Cell adhesion receptors play a fundamental role in integrating the extracellular matrix with cell signalling complexes, and thereby control diverse, distal events in metazoa such as cell fate and tissue structure. Within cells, adhesion signalling occurs at focal sites and involves the formation and maturation of discrete adhesion complexes. The sequential modulation of Rho family GTPase activity is a critical control point determining the efficacy of adhesion signalling. Adhesive responses to the extracellular matrix protein, fibronectin (FN), which are mediated by members of the integrin and syndecan adhesion receptor families, have served as a prototype for many of these studies, and the outcomes are generally applicable to a large majority of adhesive responses. Interestingly, cells plated onto a FN fragment that binds the integrin $\alpha5\beta1$ are able to spread but fail to form adhesion complexes or fully organise actin into bundled stress fibres unless co-stimulated with a distinct FN fragment that binds syndecan-4. Engagement of syndecan-4 in such pre-spread cells recapitulates the Rho family activation profiles observed during spreading on whole FN. We have found that adhesion to a ligand of $\alpha5\beta1$ alone does not activate one member of the Rho family, Rac1, indicating that engagement of syndecan-4 is an absolute requirement for this key signalling event. In related work, we have examined differences in the mechanism of adhesion complex formation mediated by different FN-binding integrins, $\alpha4\beta1$ and $\alpha5\beta1$. Two signalling differences were found. First, while $\alpha5\beta1$ required a proteoglycan co-receptor (syndecan-4), $\alpha4\beta1$ did not. Second, adhesion contact formation via $\alpha5\beta1$ required PKCα activation, but only basal PKCα activity was observed following adhesion via $\alpha4\beta1$. These findings demonstrate that different integrins can signal to induce focal adhesion formation and migration by different mechanisms, and provide insights into the ways that the extracellular environment controls cell morphology and movement.

12

NOVEL GENETIC MARKERS OF OSTEOARTHRITIS

John Loughlin

Early cross-sectional and longitudinal studies demonstrated familial clustering of primary osteoarthritis, implying a genetic component to the disease. However, such clustering could also be the result of shared environmental factors within a family. Twin studies have since been performed that demonstrate a clear heritability to OA at a number of skeletal sites, including hands, hips, knees and the spine [1]. Other epidemiological studies have also been performed investigating the nature of OA transmittance from parents to offspring and the prevalence of disease between relative-pairs, particularly siblings. These studies have confirmed a major genetic component to OA, which is transmitted in a non-Mendelian, complex manner. It has gradually become apparent that the nature of the genetic risk is likely to vary somewhat between different skeletal sites and may also vary between the sexes, although this latter observation is based on a small number of studies and needs further investigation to confirm it's veracity.

With a genetic component established the next step was a hunt for the risk alleles. Investigators initially focussed on genes encoding the major structural components of the cartilage extracellular matrix, such as aggrecan and type II collagen. These studies did not provide the expected breakthroughs and prompted a re-think on the nature of OA susceptibility: instead of the cartilage matrix being poorly constructed could the susceptibility be